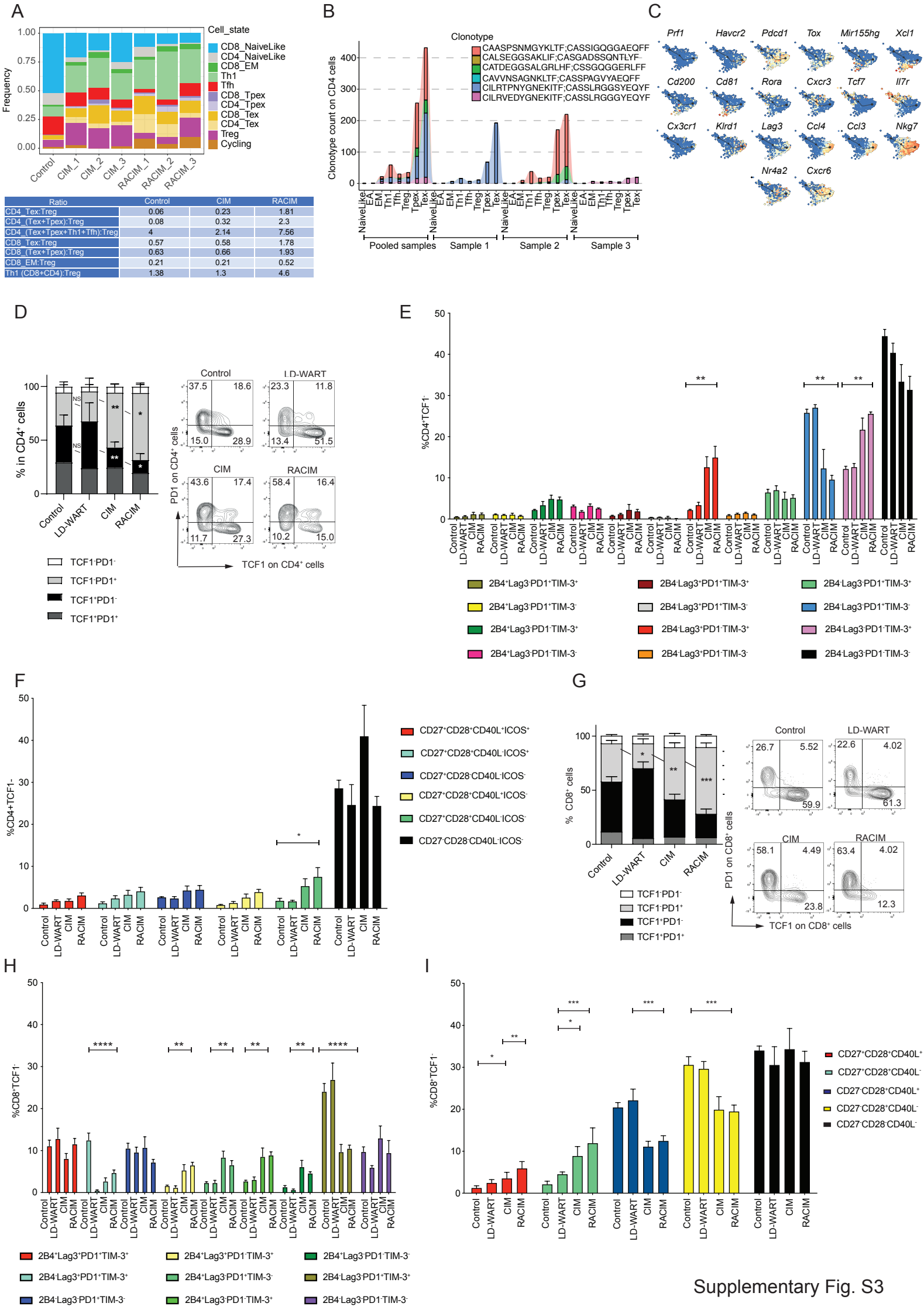

Ratio	Control	CIM	RACIM
CD4_Tex:Treg	0.06	0.23	1.81
CD4_(Tex+Tpex):Treg	0.08	0.32	2.3
CD4_(Tex+Tpex+Th1+Tfh):Treg	4	2.14	7.56
CD8_Tex:Treg	0.57	0.58	1.78
CD8_(Tex+Tpex):Treg	0.63	0.66	1.93
CD8_EM:Treg	0.21	0.21	0.52
Th1 (CD8+CD4):Treg	1.38	1.3	4.6

Supplementary Fig. S3

Supplementary Fig. S3: Effect of therapy on $CD4^+$ and $CD8^+$ T cells infiltrating ID8 tumors.

(A) Frequency of T-cell subsets by scRNAseq; precursor exhausted (Tpex), terminal exhausted (Tex), effector memory (EM), T helper 1 (Th1), T follicular helper (Tfh), regulatory T cell (Treg), and naïve like T cells in all three control tumors vs individual tumors (#1-3) from CIM or RACIM. Table describes CD8 and CD4:Treg ratios. **(B)** Bar plots representing the most clonally expanded $CD4^+$ T-cell clonotypes (by TCRseq) following RACIM (TCRs of all 3 tumors are shown in #1, *vs.* in individual tumors in #2-4). **(C)** Pseudotime trajectory analysis of CD4 clusters (as in Fig. 4F) showing the trajectory and gradual up/downregulation of indicated genes. **(D-E)** Immune cell phenotypes were evaluated on single cell suspensions of ID8 tumors treated with LD-WART, CIM or RACIM and control ID8 tumors. **(D)** Representative flow cytometry bar plot and gating strategy of $TCF1^{-/+}PD1^{+/-}$ $CD4^+$ TILs. **(E)** Flow cytometric evaluation of co-expression of indicated inhibitory receptors on $TCF1^-PD1^+CD4^+$ TILs. **(F)** Histogram representing flow cytometry evaluation of cell-surface coexpression of indicated costimulatory receptors by $TCF1^-PD1^+CD4^+$ cells. **(G)** Flow cytometry evaluation $TCF1^{-/+}PD1^{+/-}$ $CD8^+$ TIL subsets and representative gating strategy. **(H)** Histogram depicting co-expression of indicated co-inhibitory receptors on $CD8^+$ $TCF1^-PD1^+$ TILs as evaluated by flow cytometry analysis. **(I)** Histogram depicting coexpression of indicated costimulatory receptors on $CD8^+TCF1^-PD1^+$ TILs as evaluated by flow cytometric analysis. Data are representative of 2 to 3 independent experiments with n=5 mice per group. Statistical analysis was performed using Student's unpaired *t*-test or Kruskal Wallis for multiple comparisons, error bars represent mean ± standard deviation. $*P \leq 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.